Using Pharmacokinetic and Viral Kinetic Modeling To Estimate the Antiviral Effectiveness of Telaprevir, Boceprevir, and Pegylated Interferon during Triple Therapy in Treatment-Experienced Hepatitis C Virus-Infected Cirrhotic Patients.

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Using pharmacokinetic and viral kinetic modeling to estimate the antiviral effectiveness of telaprevir, boceprevir and Peg-IFN during triple therapy in treatment-experienced HCV infected cirrhotic patients (ANRS CO20-CUPIC)

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Running Head: Effectiveness of triple therapy in cirrhotic patients

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Abstract

Background Triple therapy combining a protease inhibitor (PI) telaprevir or boceprevir, pegylated-interferon (Peg-IFN) and ribavirin (RBV) have dramatically increased the chance to eradicate hepatitis C virus (HCV). However the efficacy of this treatment remains suboptimal in cirrhotic experienced-patients. Here we aimed to better understand the origin of this impaired response by estimating the antiviral effectiveness of each drug.

Methods Fifteen genotype 1-patients with compensated cirrhosis, non-responders to a prior Peg-IFN/RBV therapy were enrolled in a non-randomized study. HCV-RNA and drug concentrations of PIs, Peg-IFN and RBV were frequently assessed in the first 12 weeks of treatment and were analyzed using a pharmacokinetics/viral kinetics model.

Results Both PIs achieved similar level of molar concentrations (P=0.5), but there was a significant difference of EC_{50} (P=0.008), leading to a larger antiviral effectiveness than boceprevir in blocking viral production (99.8% *vs* 99.0%, respectively, P=0.002). In all patients the antiviral effectiveness of Peg-IFN was modest (43.4%) and there was no significant contribution of RBV exposure on the total antiviral effectiveness. The second phase of viral decline, which is attributed to the loss rate of infected cells, was slow (0.19 day^{-1}) and was higher in patients that subsequently eradicated HCV (P=0.03).

Conclusion Both PIs achieved a high level of antiviral effectiveness. However the suboptimal antiviral effectiveness of Peg-IFN/RBV and the low loss of infected cells suggest that longer treatment duration might be needed in cirrhotic treatment experienced-patients and that future IFN-free regimen may be particularly beneficial to these patients.

Keywords: Hepatitis C virus; Non-linear mixed effect models; Early viral kinetics; Protease inhibitor; Pegylated-interferon; Ribavirin; Mathematical modeling; Pharmacokinetic

Introduction

Chronic infection with hepatitis C virus (HCV) affects approximately 160 million people worldwide (1) and is the leading cause of cirrhosis, liver cancer and liver transplantation (2). The goal of treatment is to achieve a sustained virological response (SVR), marker of viral eradication, assessed by the absence of detectable HCV RNA six months after treatment discontinuation. The approval in 2011 of two protease inhibitors (PI), telaprevir and boceprevir, in combination with pegylated-interferon-alpha and ribavirin (Peg-IFN/RBV) (3), has marked an important milestone with SVR rates higher than 70% in HCV genotype 1 infected patients (4, 5). Recently two new triple therapy involving sofosbuvir, a nucleoside polymerase inhibitor, and simeprevir, a new protease inhibitor, have been approved by the European and American regulatory agencies, showing in clinical trials even higher SVR rates of 90% (6). However the cost of these new treatments, about twice as much as telaprevir or boceprevir-based therapy (7), will make them out of reach for many countries. Therefore triple therapy with Peg-IFN, RBV and telaprevir/boceprevir will continue to be vastly used in the next years and will remain the only therapeutic option for many patients.

Although these results suggest that a functional cure might be obtained in a large majority of patients, one should keep in mind that issues remain. In particular the proportion of patients with advanced liver disease and cirrhosis and/or who had failed a previous treatment with Peg-IFN/RBV is under represented in the patient population in clinical trials (8–11). The evaluation of the triple therapy in this population was precisely the goal of the ANRS-CO20-CUPIC cohort (Compassionate Use of Protease Inhibitors in viral C Cirrhosis; ClinicalTrials.gov number: NCT01514890) (12), where 511 genotype 1 treatment-experienced cirrhotic patients were included. In this study the SVR rates 12 weeks after treatment discontinuation (SVR12) were equal to 52% and 43% in telaprevir and boceprevir treated patients, respectively (13). The origin of this impaired response might encompass a

variety of factors, in particular impaired drug pharmacokinetics (PK) or limited sensitivity to PI agents and/or Peg-IFN/RBV in this particular population.

One way to evaluate treatment antiviral effectiveness and to optimize therapy is to use PK-viral kinetic (VK) models that provide a useful tool to quantitatively describe the relationship between drug exposure and viral response (reviewed in (14)). However no such analysis has been published with boceprevir and results published for telaprevir were mostly based on treatment naive and/or non-cirrhotic patients (15–17).

Here, we aimed to get new insights into the determinants of the response to triple therapy by analyzing in details, within a subset of 15 patients enrolled in the ANRS-CO20-CUPIC study, the relationship between drug concentrations and early virological response. We used the techniques of PK-VK modeling in order to tease out the relative antiviral effectiveness of each of the agents involved in the triple therapy (*i.e.*, boceprevir or telaprevir, Peg-IFN and RBV) and to investigate for a possible association with long term virological response.

Materials and methods

Patients and data

MODCUPIC is a substudy of the French multicentre prospective ANRS-CO20-CUPIC cohort. In four centres, from September 2011 to September 2012, patients chronically monoinfected with HCV genotype 1, compensated cirrhosis (Child-Pugh class A), non-responders to a prior IFN-based therapy and who started triple therapy were recruited. The diagnosis of cirrhosis was made by liver biopsy or non-invasive tests, Fibrotest® or Fibroscan® or Fibrometer® or Hepascore® at the discretion of the investigator, according to the French recommendations (18). The choice between TVR- or BOC-based therapies was at the investigator's discretion without randomization. TVR-based therapy included 12 weeks of telaprevir (750 mg/8 hours) in combination with Peg-IFN-α2a (180 µg/week) and RBV (1,000 or 1,200 mg/day, depending on body weight) then 36 weeks of Peg-IFN-α2a/RBV (named group telaprevir in the following). BOC-based therapy included 4 weeks (lead-in phase) of Peg-IFN-α2b (1.5 µg/kg/week) or Peg-IFN-α2a (180 µg/week) and RBV (800 or 1,400 mg/day, depending on body weight) then 44 weeks of Peg-IFN-α2b/RBV and boceprevir (800 mg/8 hours) (named group boceprevir in the following). Patients were followed up to six months after treatment discontinuation to assess SVR.

Written informed consent was obtained before enrolment. The protocol was conducted in accordance with the Declaration of Helsinki and was approved by the "Ile-de-France IX Ethics Committee" (Créteil, France).

Bioanalytical methods

HCV RNA and drug concentrations were measured post PIs initiation at hours 0, 8, days 1, 2, 3 and weeks 1, 2, 3, 4, 8 and 12. Patients treated with boceprevir had two additional VL and concentrations measurements during the lead-in phase. Blood samples were collected early in

121 the morning before the first daily dose of PIs and RBV and therefore only trough pre-dose
122 drug concentrations were collected. All samples were collected on SST (serum) vacutainers,
123 kept at 4°C until centrifuged at 3,000 RPM for 10 minutes in a 4°C centrifuge, within 1 hour
124 after collection, aliquoted and kept at -80°C until analysis.
125 PIs concentrations in serum were determined using ultra-performance liquid chromatography
126 coupled with tandem mass spectrometry with a lower limit of quantification (LOQ) of 5 ng/ml
127 and 10 ng/ml for boceprevir and telaprevir, respectively (19). PI concentrations were
128 converted to µmol/l for analysis using molar masses of 519.68 g/mol and 679.85 g/mol for
129 boceprevir and telaprevir, respectively. RBV concentrations in serum were determined using
130 ultra-performance liquid chromatography coupled with UV detection with a LOQ of 100
131 ng/ml (20). Peg-IFN-α2a and -α2b in serum were determined with a bioassay which was
132 chosen because the objective was to quantify the antiviral activity of Peg-IFN-α and not only
133 the concentration. Immunoassay measures the physical quantity of material but does not
134 differentiate between active and inactive molecules while bioassay for IFN-α is based on the
135 protection of cultured cells against the cytopathic effect of a challenge virus and also was
136 suitable for assaying both Peg–IFN-α-2a and Peg–IFN-α-2b. The reference solutions
137 contained 2.8–180 ng/ml of Peg-IFN-α2a (Roche Diagnostics, Germany) (21).
138 HCV-RNA levels were measured with a real-time PCR-based assay, Cobas®
139 Ampliprep/Cobas TaqMan® assay (Roche Diagnostics, Germany), with a lower limit of
140 detection (LOD) of 15 IU/ml. DNA samples were genotyped for the IL28B rs12979860
141 polymorphism (AmpliTaq gold® DNA polymerase and BigDye® terminator cycle
142 sequencing kit, Applied Biosystems, UK).
143
144 **Drug pharmacokinetic modeling**

All drug concentrations were fitted separately in telaprevir and boceprevir treatment groups. For both Peg-IFN and RBV, the trough serum concentrations, noted $C^{Peg\text{-}IFN}(t)$ and $C^{RBV}(t)$, respectively were fitted using an exponential model to reflect the progressive increase in trough drug concentrations over time:

$$C^{Peg-IFN}(t) = C_{ss}^{Peg-IFN} \times (1 - e^{-kt}) \quad \text{Eq. (1)}$$

$$C^{RBV}(t) = C_{ss}^{RBV} \times (1 - e^{-kt}) \quad \text{Eq. (2)}$$

where C_{ss} is the trough concentration at steady state and k the rate constant of elimination which reflects the progressive increase in $C(t)$ over time.

For both PI drugs, consistent with the fact that they have a short elimination half-life (22), no significant increase of trough concentrations over time was observed. Therefore concentrations for both telaprevir and boceprevir were fitted using a constant model, where C_{ss} is the trough concentration:

$$C^{PI}(t) = C_{ss}^{PI} \quad \text{Eq. (3)}$$

Viral kinetic modeling

The following model of HCV viral kinetics (VK) was used to fit the changes in HCV RNA (23):

$$\frac{dI}{dt} = bVT - \delta I$$

$$\frac{dV}{dt} = p\big(1 - \varepsilon(t)\big)I - cV \quad \text{Eq. (4)}$$

where T represent the target cells that can be infected by virus, V, with rate b. Infected cells, I, are lost with rate δ and produce p virions per day, which are cleared from serum with rate c. The target cell level is assumed constant throughout the study period (12 weeks) and remains at its pre-treatment value $T_0=c\delta/p\beta$. Treatment is assumed to reduce the average rate of viral production per cell from p to $p(1–\varepsilon)$, where ε represents the drug antiviral effectivenesses, *i.e.*, $\varepsilon = 0.99$ implying the drug is 99% effective in blocking viral production. This model predicts

that VL will fall in a biphasic manner, with a rapid first phase lasting for a couple of days that reduce the VL with a magnitude equal to $\log_{10}(1-\varepsilon)$, followed by a second slower but persistent second phase of viral decline with rate $\varepsilon\delta$. Therefore a difference between ε = 99.9% and ε = 99.0% corresponds to a 10-fold difference in the viral production under treatment and will lead to 1-log difference between the two curves of viral decline (24). We fixed p and b to 100 IU/ml/cell/day and 10^{-7} $(IU/ml)^{-1}$/day, respectively, without loss of generality (25).

The effectiveness of each drug in blocking viral production was described by an E_{max} model assuming a maximum inhibition of 100%:

$$\varepsilon^{PI}(t) = \frac{C^{PI}(t)}{C^{PI}(t)+EC_{50}^{PI}}$$

$$\varepsilon^{Peg-IFN}(t) = \frac{C^{Peg-IFN}(t)}{C^{Peg-IFN}(t)+EC_{50}^{Peg-IFN}} \quad \text{Eq. (5)}$$

where EC_{50}^{PI} (respectively $EC_{50}^{Peg\text{-}IFN}$) is the PI (resp. Peg-IFN) concentration at which the PI (resp. Peg-IFN) is 50% effective, and $C^{PI}(t)$ (resp. $C^{Peg\text{-}IFN}(t)$) are the individual predictions (see below) given by the PK models (Eq. 1 and 3).

The combined effect of PIs and Peg-IFN was modeled using a Bliss independent action model (26) and the total efficacy $\varepsilon(t)$ was given by:

$$\left(1-\varepsilon(t)\right) = (1-\varepsilon^{PI}(t))(1-\varepsilon^{Peg-IFN}(t)) \quad \text{Eq. (6)}$$

Since the effect of RBV on the early virological response is expected to be modest (27–29) we did not incorporate the effect of RBV into the reference model (Eq. 4-6). In a second step we tested whether the effectiveness of RBV, also modeled using an E_{max} model could enhance the effect in blocking viral production or reduce viral infectivity, as suggested previously (30).

Data analysis and parameter estimation

The pharmacokinetics/viral kinetics (PK-VK) model given by Eq. 4-6 can be used only to characterize the viral kinetics of drug sensitive virus and therefore cannot fit viral rebounds due to the emergence of drug-resistant virus. Therefore only HCV RNA data until virologic rebounds (with no indication of lack of compliance) were used to estimate the viral kinetic parameters.

Parameters V_0, c, δ, EC_{50}^{PI} and $EC_{50}^{Peg\text{-}IFN}$ were estimated using non-linear mixed-effect models (NLMEM). In this approach, each individual parameter θ_i is comprised of a fixed part θ, which represents the mean value of the parameter in the population (fixed effects), and a random part η_i chosen from a Gaussian distribution with mean 0 and standard deviation ω_i that accounts for the inter-individual variability. Therefore, for all parameters $\theta_i = \theta e^{\eta_i}$ where $\eta_i \sim N(0, \omega^2)$. Both PK data and Log_{10}(HCV RNA) were best described using an additive residual error with constant variance.

Model parameters were estimated using the Stochastic Approximation Expectation Minimization (SAEM) algorithm in MONOLIX v4.2 (available at http://www.lixoft.eu). Of note this approach is based on maximum likelihood estimation which take into account the information brought by data under the LOD as left-censored data (31, 32).

Model selection was done using the Bayesian information criteria (BIC), a fitting criterion derived for each model from the computation of likelihood that takes into account the number of estimated parameters used (the lower the better (33)). Model evaluation was performed using goodness-of-fit plots, as well as the individual weighted residuals (IWRES) and the normalized prediction distribution errors (NPDE) over time.

Difference in PK-VK model parameters between telaprevir and boceprevir treatment group

A Wald test on the PK-VK model parameters (c, δ, EC_{50}^{PI}) was used to assess the difference in population parameters between the two groups. Because we previously showed that this approach could lead to an inflation of the type I error in case of small sample size (N<20 per group) (34), a permutation test was performed to confirm statistical significance when the Wald test was significant at the level of 5%. In brief, 1,000 datasets were simulated by randomly allocating patients to telaprevir or boceprevir group, maintaining a similar proportion of patients allocated to each groups than in the original dataset. Then the P-value of the Wald test was calculated for each simulated data set. Finally the corrected P-value of the permutation test is equal to the proportion of simulated datasets having a P-value lower than the one found one the original dataset.

Because the genetic barrier to resistance of PI (*i.e.*, the number of change in amino acids needed to generate mutants with high level of resistance) depends of HCV subgenotype and therefore lead to different SVR rate, we also estimated the effect of HCV subgenotype (1a *vs* non-1a) on viral kinetic parameters. IL28B polymorphism, which is also associated with response to IFN-based therapy, was not investigated because all these patients had failed to a previous bitherapy.

Prediction and comparison of individual parameters

Individual Empirical Bayesian Estimates (EBE) parameters for both PK and VK were obtained by computing for each patient the Maximum A Posteriori (MAP) estimate. The individual antiviral effectiveness at steady state, ε_{ss}, of each agent was defined by:

$$\varepsilon_{ss}^{PI} = \frac{C_{ss}^{PI}}{C_{ss}^{PI}+EC_{50}^{PI}}$$

$$\varepsilon_{ss}^{Peg-IFN} = \frac{C_{ss}^{Peg-IFN}}{C_{ss}^{Peg-IFN}+EC_{50}^{Peg-IFN}} \quad \text{Eq. (7)}$$

240 Non-parametric two-sided tests (Wilcoxon test) were used to compare i) individual EBE PK
241 parameters between patients who received telaprevir *vs* boceprevir and between patients who
242 received Peg-IFN-α2a *vs* -α2b, and ii) individual EBE PK parameters between SVR and non-
243 SVR patients. Because all patients were non-responder to Peg-IFN, the effect of IL28B
244 genotype on PK and VK parameters was not tested.
245

Results

Fifteen HCV genotype 1 patients were included 9 receiving telaprevir and 6 receiving boceprevir. Twelve (80%) were men, with a median [min; max] age of 55 [44; 64] years. Seven (47%) patients were infected with subgenotype 1a, 2 (22%) in telaprevir group and 5 (83%) in boceprevir group. Prior treatment responses were partial response, null response, relapse and early discontinuation for adverse events in 2, 5, 6 and 2 patients, respectively. Only two patients had the most favorable IL28B CC genotype (35). Main characteristics of the patients are presented in Table 1.

Two patients had a viral breakthrough (at weeks 3 and 8). Eleven patients received Peg-IFN-α2a (8 in telaprevir group and 3 in boceprevir group), 3 patients Peg-IFN-α2b (all in boceprevir group) and one patient in telaprevir group did not receive any injection of Peg-IFN (and this patient had a viral breakthrough at week 3).

Fig. 1 shows the observed drug concentrations versus time and Table 2 gives the estimated steady state trough concentrations, C_{ss}, for all drugs. There was no significant difference in the molar medians steady state concentrations of telaprevir and boceprevir ($C_{ss}^{telaprevir}$ = 3.77 [2.68; 5.98] µmol/l *i.e.* 2,563.0 ng/ml [1,822.0; 4,065.5] and $C_{ss}^{boceprevir}$ = 3.92 [3.22; 7.64] µmol/l *i.e.* 2037.1 ng/ml [1,673.4; 3,970.4], P=0.5). There was no significant difference in the median steady state concentrations of Peg-IFN-α2a and -α2b ($C_{ss}^{Peg\text{-}IFN\text{-}2a}$ = 89.6 [52.8; 110.4] ng/ml and $C_{ss}^{Peg\text{-}IFN\text{-}2b}$ = 55.4 [55.3; 57.9] ng/ml, P=0.2). The concentrations of RBV increased over time in all patients and could be well captured by our model (Eq. 2) with a median k equal to 0.10 day^{-1}, corresponding to a half-life of increase of about 7 days. At equilibrium medians C_{ss}^{RBV} were equal to 2,860 [2,428; 3,874] ng/ml.

After the PK parameters were estimated, the predicted individual PK time courses were plugged into the PK-VK model (see methods). Baseline VL was higher in the telaprevir group than in the boceprevir group, thus a treatment group effect was added on baseline VL

($V_0^{telaprevir}$ = 6.43 $\log_{10}$ IU/ml *vs* $V_0^{boceprevir}$ = 5.52 $\log_{10}$ IU/ml, P=0.0001). A greater proportion of patients that received boceprevir were genotype 1a relative to those that received telaprevir (P=0.04). Subgenotype is an important predictor of the response to treatment, in particular with telaprevir with a lower genetic barrier to resistance with genotype 1a than 1b (only one nucleotide change in genotype 1a viral genomes is required to generate mutations V36M and R155K/T, *vs* two in genotype 1b) (36). This may explain why genotype 1a patients were preferentially treated with boceprevir. We did not find any significant effect of subgenotype on any of the parameters.

The model could well describe the kinetics of HCV decline observed both during the lead-in phase (in the boceprevir group) and after the initiation of the PIs (in both groups, see Fig. 2). There was no evidence of model misspecification as showed by the goodness-of-fit plot (Fig. 3) and all parameters could be estimated with a good precision (Table 3).

The model predicted a mean $EC_{50}^{Peg\text{-}IFN}$ equal to 106 ng/ml, leading to a low antiviral effectiveness at steady state of Peg-IFN at steady state of 43.4% [0.0; 52.7], consistent with the modest 0.67 $\log_{10}$ IU/ml drop observed during the four weeks lead-in phase in patients treated with boceprevir (Fig. 2).

After PI initiation, VL declines in a biphasic manner in all patients, where a rapid first phase was followed by a second slower phase. The rapid first phase was attributed to a clearance rate of virus, c, equal to 3.98 day^{-1} and to a high level of antiviral effectivenesses for both PIs. The intrinsic potency of the two molecules, as measured by the EC_{50}^{PI}, was significantly higher for telaprevir than boceprevir ($EC_{50}^{telaprevir}$ = 0.009 µmol/l *vs* $EC_{50}^{boceprevir}$ = 0.04 µmol/l, P=0.008). Importantly the statistical significance of this difference was obtained after taking into account the small sample size (see methods) and adjusted on baseline VL. Since telaprevir had a lower EC_{50} than boceprevir and that both drugs achieved similar levels of molar concentrations the model predicted that the median individual antiviral effectiveness of

PI agent in blocking viral production was significantly higher in patients that received telaprevir than in those who received boceprevir ($\varepsilon_{ss}^{telaprevir}$ = 99.8% [99.3; 99.9] and $\varepsilon_{ss}^{boceprevir}$ = 99.0% [98.0; 99.6], P=0.002). Interestingly this model could well capture the relationship between the serum exposure and its antiviral effectiveness, demonstrating that the variability in drug exposure needs to be taken into account to understand the between-subject variability in PIs antiviral effectiveness (Fig. 4A). Lastly because the effectiveness of both PIs were much larger than that of Peg-IFN (Fig. 4B), the total antiviral effectiveness obtained by the combination of PI and Peg-IFN was largely similar to the one obtained with the PIs only.

After the VL was rapidly reduced as a result of the strong antiviral effectiveness of both PIs, the model predicted that a second slower phase of viral decline ensued, driven by the loss rate of infected cells, δ. We estimated δ to be equal to 0.18 day^{-1}, corresponding to a half-life of infected cells of 3.9 days, with no significant differences between patients receiving telaprevir and boceprevir (P=0.5).

Next we investigated the relationship between the PK-VK parameters and SVR. Among the 7 patients (47%) who achieved SVR, 5 received telaprevir and 2 received boceprevir (56% *vs* 33%, respectively, P=0.6). As shown in Fig. 5, neither the antiviral effectivenesses of PIs nor that of Peg-IFN was significantly associated with the long term virological response. However the loss rate of infected cells, δ, was significantly higher in patients that subsequently achieved SVR (median δ^{SVR} = 0.27 day^{-1} *vs* median $\delta^{non\text{-}SVR}$ = 0.14 day^{-1}, P=0.03).

Lastly we verified that incorporating the effect of RBV exposure in the PK-VK model, either on the block of viral production or in the decrease of viral infectivity (data not shown) did not improve the fit of the data. Furthermore there was no significant association between the predicted C_{ss}^{RBV} and long term virological response (P=0.5).

Discussion

Here we used a PK-VK model to provide the first detailed picture of the relationship between the exposure to all drugs involved in triple therapy (Peg-IFN, RBV and telaprevir or boceprevir) and the early virological response. This novel model provides important insights into the understanding of the response to triple therapy in hard-to treat patients.

We predicted that both PIs achieved a high level of antiviral effectiveness in blocking viral production that was higher than 97.9% in all patients. However telaprevir had a higher intrinsic potency than boceprevir, as measured by EC_{50} (P=0.008 after correcting for small sample size), leading to a significantly higher level of antiviral effectiveness than boceprevir ($\varepsilon_{ss}^{telaprevir}$ = 99.8% *vs* $\varepsilon_{ss}^{boceprevir}$ = 99.0%, P=0.002) *i.e.* a 5-fold difference in the viral production under treatment. Importantly the difference in EC_{50} was obtained despite the fact that the study was not randomized and that patients who received telaprevir had less favorable baseline characteristics than those who received boceprevir with higher baseline VL (6.43 $\log_{10}$ IU/ml *vs* 5.52 $\log_{10}$ IU/ml, respectively, $P<10^{-4}$) and a higher proportion of null responder to previous bitherapy (4/9 *vs* 1/6).

The comparison of drug's antiviral effectiveness should be taken with caution because of small sample size, the absence of randomization, and the fact that only trough concentrations were used to estimate the EC_{50} of PI which may lead to underestimation. Yet these results demonstrate for the first time a significant association between serum exposure to PI agents and the antiviral effectiveness achieved. To confirm the significance of this association we fitted HCV RNA data to a simplified model where drug exposure was not taken into account (37). As compared to this model, we found that the PK-VK model both improved the fitting criterion (BIC decreases from 181.3 to 176.3, *i.e.* an improvement of 5 points which is regarded as positive evidence) and reduced the between-patient parameter variability by 26%

($\omega_{EC_{50}PI}$ from 0.85 to 0.61), thus demonstrating that serum PK is an important predictor of the antiviral effectiveness of triple therapy.

Our estimate that telaprevir achieves an antiviral effectiveness of 99.8% is largely similar to the one found in naïve patients (15), suggesting that compensated cirrhosis does not affect the maximal antiviral effectiveness of telaprevir. Whether this is also true for boceprevir is not known as to our knowledge there is no published viral kinetic modeling study evaluating the *in vivo* antiviral effectiveness of boceprevir.

In contrast to the high effectiveness achieved by both PIs, Peg-IFN was found to have a modest contribution in blocking viral production, with a mean value of 43.4%. Of note including the patient who did not receive Peg-IFN in our analysis allow us to add information on telaprevir antiviral effectiveness. Further RBV exposure had no significant contribution on the early viral kinetics. Together these results indicate that Peg-IFN and RBV have a minimal contribution on the early virologic response, at least on this population of previous non-responders to a Peg-IFN/RBV therapy.

In order to achieve a rapid viral decline, it is important to achieve not only a high level of effectiveness but also a rapid second phase of viral decline. Here the latter was rather slow in both treatment groups compared to what had been than found in telaprevir treated patients, and this was attributed in our model to a low loss rate of infected cells, δ, about three times smaller than in non-cirrhotic naive-patients (δ of 0.18 day^{-1} *vs* 0.60 day^{-1}) (15, 16). Those lower values may encompass several factors, such a lower penetration of PIs into infected cells in a highly scarced liver. Because the loss rate of infected cells is strongly related to the treatment duration needed to achieve SVR (15), our results suggest that the time to achieve SVR in this population could be longer than what had been predicted from clinical trials (15). Consistent with this prediction, the relapse rate in the CUPIC trial was equal to 41% in both

368 treatment groups (13), *i.e.*, much higher than what reported in treatment experienced patients
369 phase 3 clinical trials (12% to 27%) (9, 11, 22).
370 Regarding the use of early viral kinetic parameters for treatment prediction, we found that δ
371 was higher in patients that subsequently achieved SVR (median $\delta^{SVR} = 0.27$ day^{-1} *vs* median
372 $\delta^{non\text{-}SVR} = 0.14$ day^{-1}, P=0.03) suggesting that δ could be a relevant predictor of the outcome of
373 triple therapy, as it was the case for Peg-IFN/RBV bitherapy (38). In contrast there was no
374 significant relationship between antiviral effectiveness of PIs on SVR (Fig. 6A). This absence
375 of relationship is consistent with the hypothesis that in order to achieve SVR, it is necessary
376 not only to have a high antiviral effectiveness at treatment initiation, when the viral
377 population is predominantly wild-type and drug-sensitive, but also at later times, when the
378 viral population is predominantly resistant to PI agents (39, 40). The fact that neither Peg-IFN
379 effectiveness nor RBV were associated with SVR is more surprising, as one would expect
380 these agents to be equally active against wild-type and resistant virus. However our patient
381 population was both treatment experienced and cirrhotic, two major causes of insensitivity to
382 Peg-IFN/RBV.
383 Clearly the main limitation of this study was its small size. In a previous study we evaluated
384 by simulation the power to detect a difference of antiviral effectiveness between two
385 treatment groups for a variety of designs (34). With a design comparable to the present study,
386 *i.e.*, 10 patients per group, 7 VL per patient and an antiviral effectiveness of 99% *vs* 99.9%,
387 the power to detect this difference was 100% with the same statistical method that we used in
388 this analysis. Yet, further studies on larger populations will still be needed to estimate more
389 precisely the exposure-effect relationship (Fig. 4) and other kinetic parameters involved on
390 the long-term virologic response. A second limitation is that only trough pre-dose drug
391 concentrations were collected and modeled. Thus C_{ss} is the steady-state C_{trough}. Moreover no
392 information was collected on treatment adherence. The data analysis did not show any signal

393 of lack of adherence such as viral oscillations, which indicates that missed doses, if they
394 occurred, did not have a major effect on the observed kinetic of decline. Here we considered
395 that concentrations of PIs were constant over time. Detailed pharmacokinetic analysis showed
396 that steady state of residual concentrations is attained after two days of treatment (41). As
397 explained in details in Guedj *et al*. (42), the fact that we neglected this initial build up may
398 explain why our estimate of the viral clearance rate, *c*, was lower than previously found in
399 treatment naïve patients (15). Further the lack of information on the time of Peg-IFN injection
400 also precluded a precise characterization between Peg-IFN exposure and the virological
401 response. The fact that we used rather empirical models is less problematic for RBV, whose
402 long elimination half-life resulting in a slow increase over time could be well characterized
403 here (27). Moreover, as mentioned previously, in order to achieve SVR, it is important for
404 drugs to achieve a higher effectiveness against PI-resistant virus. Because no sequencing was
405 done here, we focused only the early virological response where presumably the virus is
406 predominantly drug-sensitive. In order to estimate PI effectiveness against resistant virus it
407 would be needed to quantify and follow the proportion of resistant virus over time, as early as
408 possible, for instance using pyrosequencing (43).
409 A greater proportion of patients that received boceprevir were genotype 1a relative to those
410 that received telaprevir (P=0.04). It has been well established that subgenotype is an important
411 predictor of the response to treatment and for instance the fact that telaprevir has a higher
412 genetic barrier to resistance with genotype 1b than 1a (36) may explain why genotype non-1a
413 patients were preferentially treated with telaprevir than boceprevir. However the effect of
414 subgenotype on the early viral kinetics, where most of the virus is drug-sensitive is unknown,
415 and has never been investigated as far as we know. In our study no significant effect of
416 subgenotype on any of the parameters (c, δ, EC_{50}^{PI}) was found.

417 The effect of RBV was analyzed using serum drug concentrations. Some authors preferably
418 used erythrocyte RBV concentration (44), which was not measured in the present study.
419 However a significant relationship was shown between erythrocyte RBV concentrations and
420 serum concentrations (45), suggesting that serum RBV can be used for the assessment of early
421 and sustained virological responses (46, 47).
422 To summarize this study provides the first characterization of the relationship between drug
423 concentrations involved in triple therapy and early HCV viral kinetics treated with telaprevir
424 or boceprevir. We found that median values of antiviral effectiveness for telaprevir was
425 similar to what had been found in treatment naïve patients and significantly larger than in
426 boceprevir treated patients. In all patients the second phase of viral decline was slow and may
427 explain the high relapse rate observed in the ANRS-CO20-CUPIC cohort. This suggests that,
428 notwithstanding safety issues, longer treatment duration could improve the treatment efficacy
429 and lead to a higher SVR rate. Lastly the antiviral effectiveness of Peg-IFN was modest (less
430 than 50%) suggesting that cirrhotic treatment experienced-patients may particularly benefit
431 from upcoming IFN-free treatment. Our approach, which shows the importance of PK data to
432 disentangle the effects of drug combination and to understand the variability in the virological
433 response, is not specific to triple therapy and could also be used to optimize future IFN-free
434 regimen, in particular in hard-to-treat patients.
435

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Author Contributions: CL, FM, and JG made the analysis and drafted the manuscript; all authors provided the data; all authors read and approved the final manuscript.

Disclosure statement

JG: has consulted with Gilead SC.

FZ: received speakers/consulting fees from Gilead SC, MSD, BMS, Janssen cilag, Abbvie, Boehringer Ingelheim.

CH: has been a clinical investigator, speaker and/or consultant for Abbvie, Boehringer Ingelheim, BMS, Gilead Sciences, Janssen, Merck Sharp & Dohme, and Roche.

PM: has been a clinical investigator, speaker and/or consultant for Roche, Gilead, Vertex, Novartis, Janssen - Tibotec, MSD, Boehringer, Abbott, Pfizer, Alios BioPharma.

GP: has received travel grants, consultancy fees, honoraria or study grants from various pharmaceutical companies, including Bristol-Myers-Squibb, Gilead SC, Janssen, Merck, ViiV Healthcare and Splicos.

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627

Figure legends

Fig. 1: Observed concentrations over time.

(a) telaprevir in 9 patients (black, µmol/ml) and boceprevir in 6 six patients (grey, µmol/ml); (b) Peg-IFN in telaprevir group (black, ng/ml) and in boceprevir group (grey, ng/ml); (c) RBV in telaprevir group (black, ng/ml) and in boceprevir group (grey, ng/ml). Patients who received a boceprevir-based therapy had only two blood samples during the lead-in phase at baseline and week 2.

Fig. 2: Individual fits of the viral decline (log_{10} IU/ml).

Nine patients in telaprevir group (black curve) and 6 patients in boceprevir group (grey curve). Black crosses represent the observed viral load and grey stars represent the viral load under the limit of detection.

Fig. 3. Goodness-of-fit of the viral kinetic-pharmacokinetic model

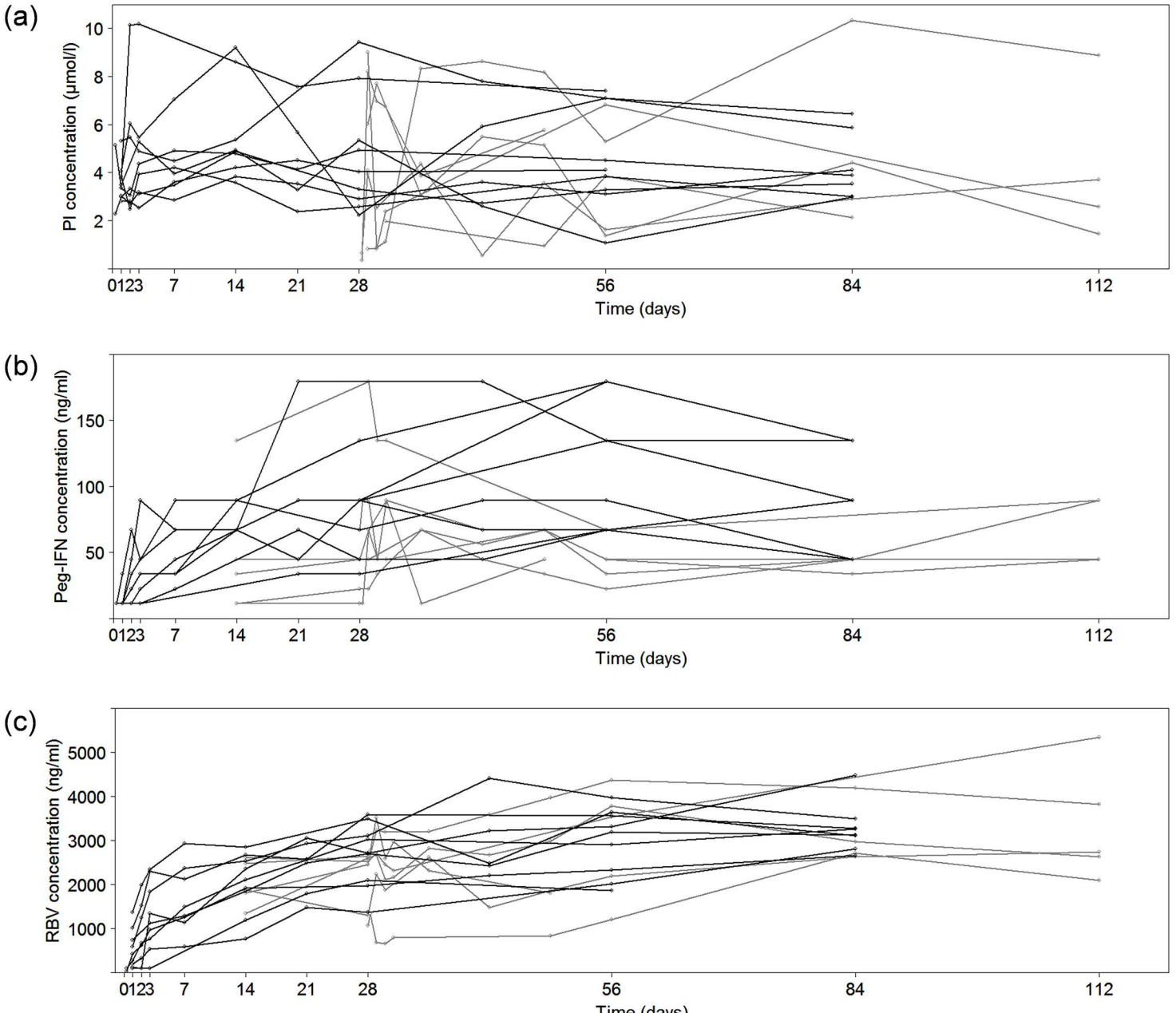
Residuals (weighted residuals calculated using individual predictions: IWRES and normalized prediction distribution errors: NPDE) versus time and versus predictions plots. Residuals seem to distribute homogenously around 0.

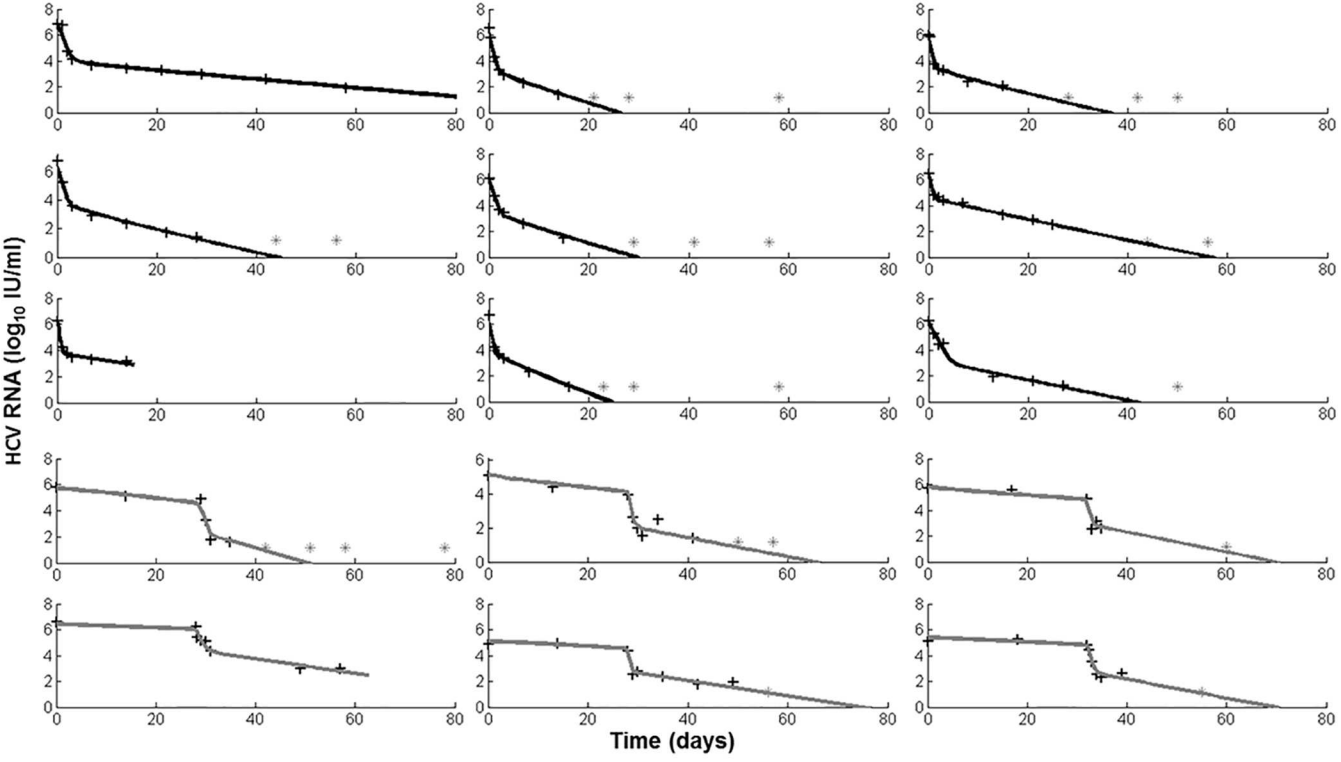
Observed viral load are plotted as black crosses and viral load under the limit of detection as grey stars.

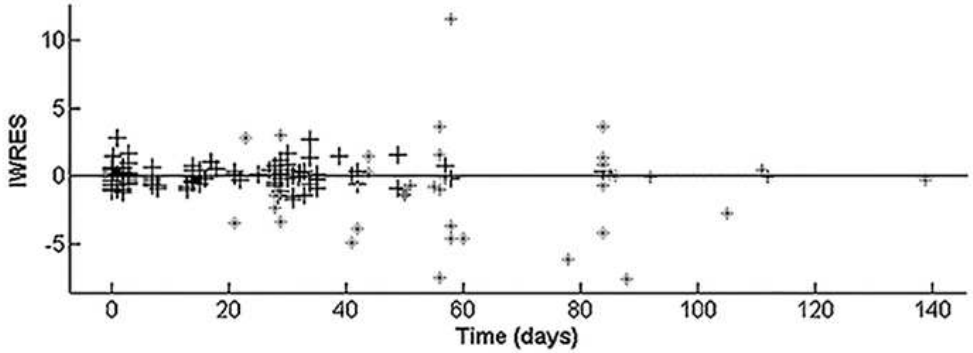
Fig. 4. Relationship between predicted trough concentration at steady state (C_{ss}) and predicted antiviral effectivenesses (ε_{ss}).

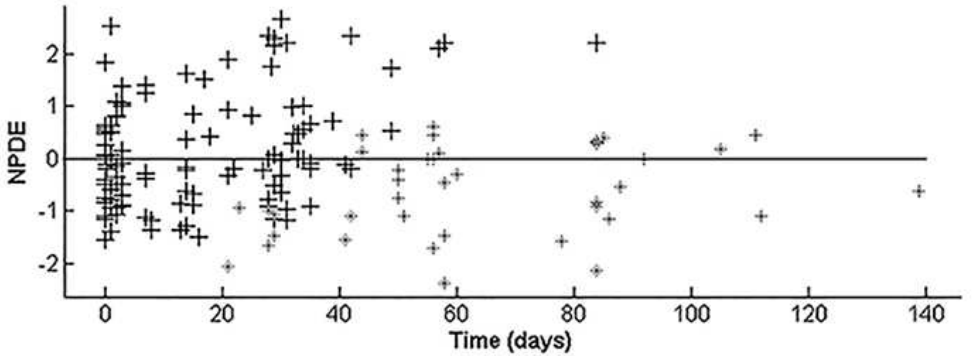
(a) for the protease inhibitor (telaprevir in black and boceprevir in grey, µmol/l); (b) for Peg-IFN (Peg-IFN-α2a in black and Peg-IFN-α2b in grey, ng/ml). The lines denote the predictions

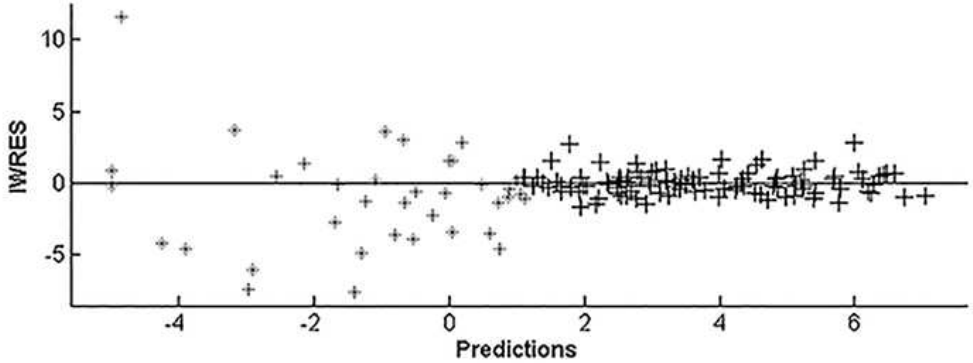
653 with the mean antiviral effectiveness and the dotted lines denote 95% confidence interval
654 computed with the standard errors predicted by the Fisher Information Matrix.
655
656 **Fig. 5: Relationship between long term virological response (SVR) and parameters**
657 **estimated by the viral kinetic-pharmacokinetic model.**
658 (a) predicted antiviral effectivenesses (ε_{ss}) of PIs; (b) predicted antiviral effectivenesses (ε_{ss})
659 of Peg-IFN; (c) δ delta parameter (loss rate of infected cells). P-value from Wilcoxon tests.

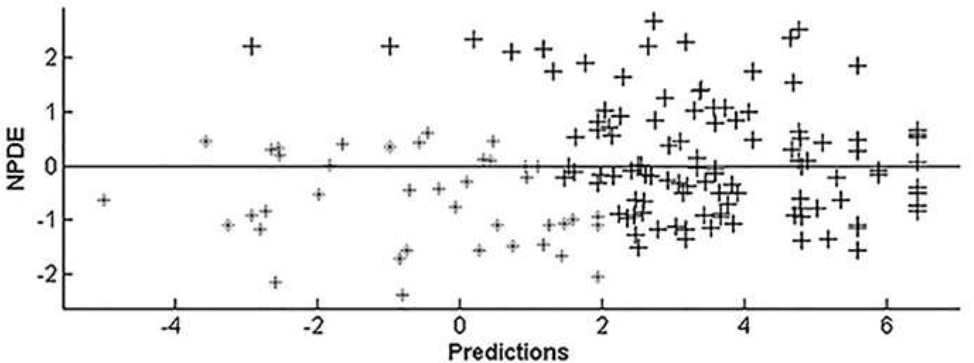

(a)
PI concentration (µmol/l)
10
8
6
4
2
0123
7
14
21
28
56
84
112
Time (days)
(b)
Peg-IFN concentration (ng/ml)
150
100
50
0123
7
14
21
28
56
84
112
Time (days)
(c)
RBV concentration (ng/ml)
5000
4000
3000
2000
1000
0123
7
14
21
28
56
84
112
Time (days)

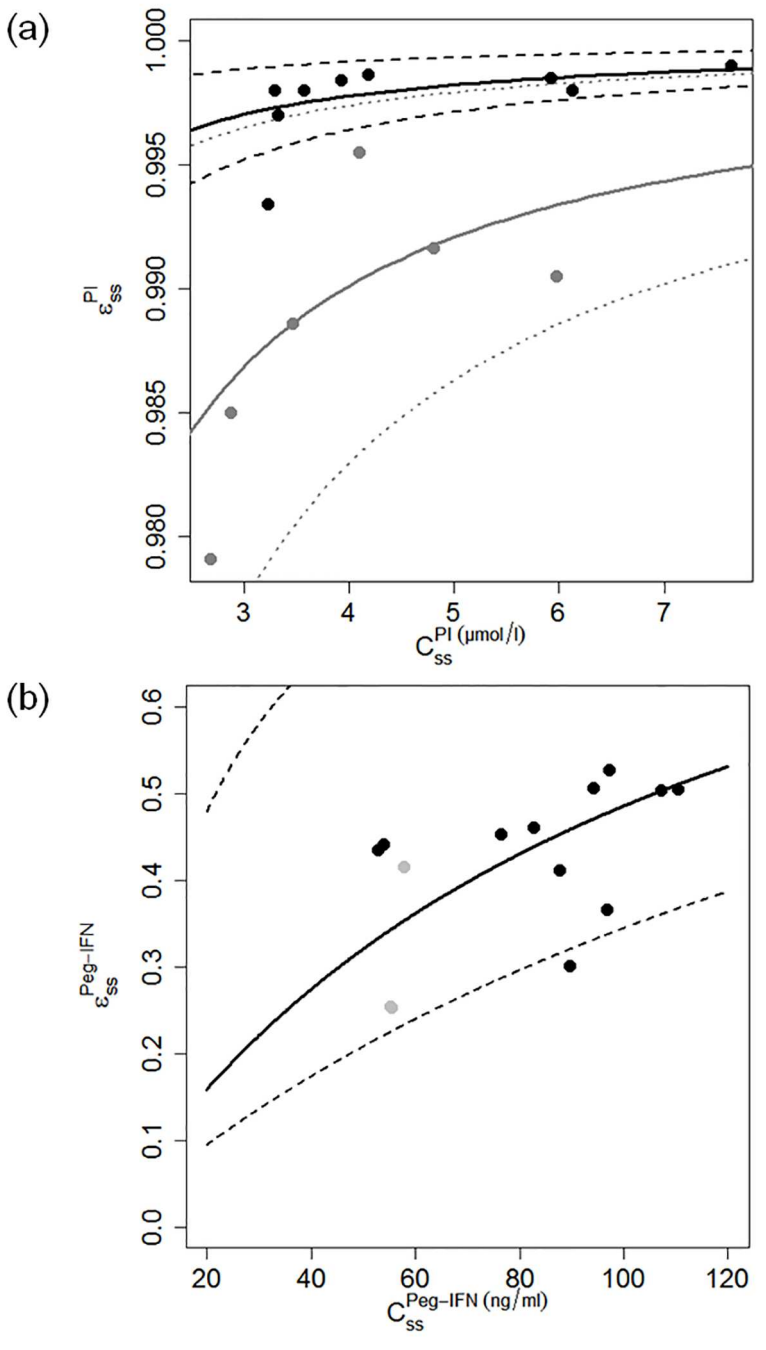

HCV RNA (log10 IU/ml)
Time (days)


IWRES
10
5
0
-5
0
20
40
60
80
100
120
140
Time (days)


NPDE
2
1
0
-1
-2
0
20
40
60
80
100
120
140
Time (days)


IWRES
10
5
0
-5
-4
-2
0
2
4
6
Predictions


NPDE
2
1
0
-1
-2
-4
-2
0
2
4
6
Predictions


(a)
1.000
0.995
0.990
0.985
0.980
ε_{ss}^{PI}
3
4
5
6
7
$C_{ss}^{PI\ (\mu mol/l)}$
(b)
0.6
0.5
0.4
0.3
0.2
0.1
0.0
$\varepsilon_{ss}^{Peg-IFN}$
20
40
60
80
100
120
$C_{ss}^{Peg-IFN\ (ng/ml)}$

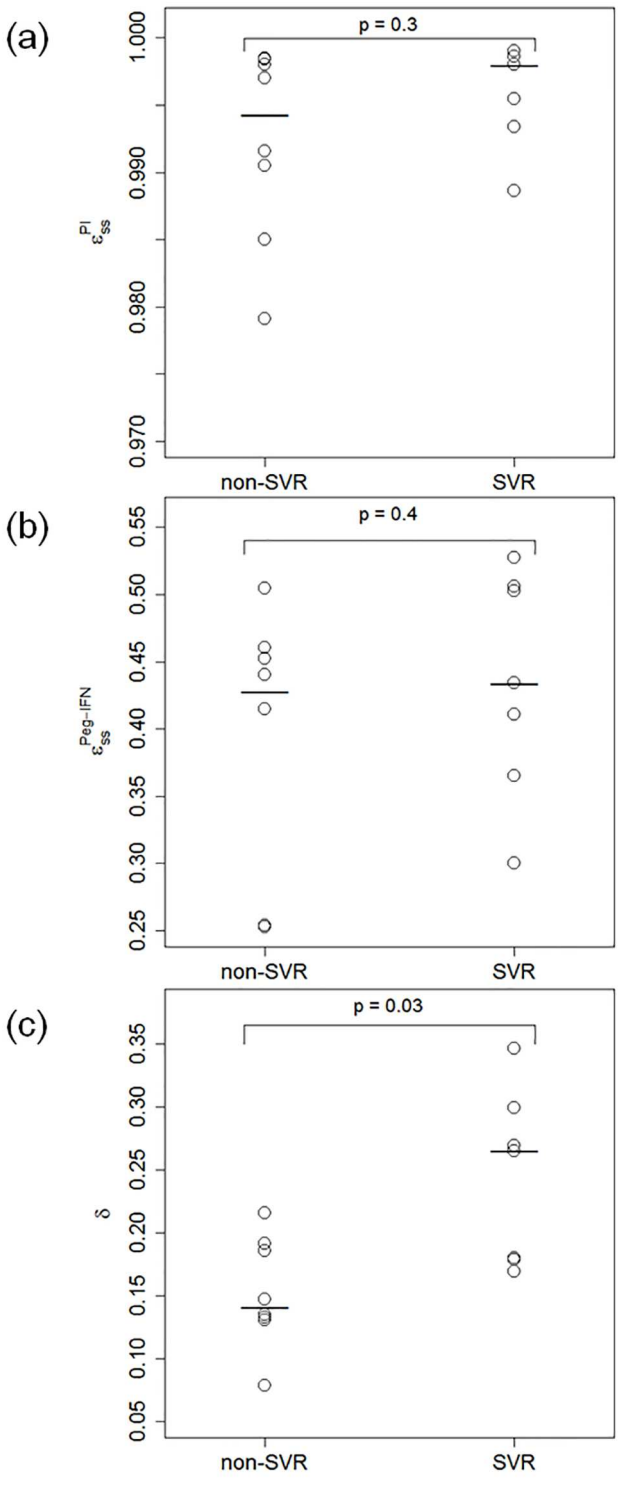

(a)
p = 0.3
1.000
0.990
0.980
0.970
ε_{ss}^{PI}
non-SVR
SVR
(b)
p = 0.4
0.55
0.50
0.45
0.40
0.35
0.30
0.25
$\varepsilon_{ss}^{Peg-IFN}$
non-SVR
SVR
(c)
p = 0.03
0.35
0.30
0.25
0.20
0.15
0.10
0.05
δ
non-SVR
SVR

Table 1. Main patient characteristics

	Peg-IFN/RBV + telaprevir n=9	Peg-IFN/RBV + boceprevir n=6	Total n=15
Age (years), median [min-max]	55 [49-59]	53 [44-64]	55 [44-64]
Males, n (%)	8 (89)	4 (67)	12 (80)
HCV RNA (log_{10} IU/ml), median [min-max]	6.5 [6.0-6.8]	5.4 [4.9-6.6]	6.2 [4.9-6.8]
HCV genotype, n (%):			
1a	2 (22)	5 (83)	7 (47)
Non 1a	7 (78)	1 (17)	8 (53)
IL28B genotype (rs12979860), n (%):			
C/C	2 (22)	-	2 (13)
C/T	6 (67)	6 (100)	12 (80)
T/T	1 (11)	-	1 (7)
Response to previous bitherapy, n (%):			
Partial responder	-	2 (33)	2 (13)
Null responder	4 (44)	1 (17)	5 (33)
Relapser	3 (33)	3 (50)	6 (40)
Early discontinuation for adverse event	2 (22)	-	2 (13)

Table 2. Individual predicted trough concentrations at steady state (C_{ss})

	n	median [min; max]
$C_{ss}^{telaprevir}$ (µmol/l)	9	3.77 [2.68; 5.98]
$C_{ss}^{boceprevir}$ (µmol/l)	6	3.92 [3.22; 7.64]
$C_{ss}^{Peg\text{-}IFN\text{-}\alpha 2a}$ (ng/ml)	11	89.6 [52.8; 110.4]
$C_{ss}^{Peg\text{-}IFN\text{-}\alpha 2b}$ (ng/ml)	3	55.4 [55.3; 57.9]
C_{ss}^{RBV} (ng/ml)	15	2,860 [2,428; 3,874]

Table 3. Parameter estimates and relative standard errors (RSE)

	Estimate	RSE (%)
$V_0^{telaprevir}$ (log_{10} IU/ml)	6.43	2
$V_0^{boceprevir}$ (log_{10} IU/ml)	5.52	3
c (day^{-1})	3.98	12
δ (day^{-1})	0.18	11
$EC_{50}^{Peg\text{-}IFN}$ (ng/ml)	106	40
$EC_{50}^{telaprevir}$ (µmol/l)	0.009	30
$EC_{50}^{boceprevir}$ (µmol/l)	0.04	43
ω_{V_0}	0.07	20
ω_c	0.47	19
ω_δ	0.42	16
$\omega_{EC_{50}}^{Peg\text{-}IFN}$	0.67	30
$\omega_{EC_{50}}^{PI}$	0.61	32
σ	0.27	7

V_0: baseline viral load; c: clearance rate of virus from serum; δ: loss rate of infected cells; EC_{50}: half maximal effective concentration; ω: inter-individual variability; σ: standard deviation of residual error; RSE: relative standard errors of parameter estimates, PI: protease inhibitor.